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Community History Affects the Predictability of Microbial Ecosystem Development

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Microbial communities mediate crucial biogeochemical, biomedical and biotechnological processes, yet our understanding of their assembly, and our ability to control its outcome, remain poor. Existing evidence presents conflicting views on whether microbial ecosystem assembly is predictable, or inherently unpredictable. We address this issue using a well-controlled laboratory model system, in which source microbial communities colonize a pristine environment to form complex, nutrient-cycling ecosystems. When the source communities colonize a novel environment, final community composition and function (as measured by redox potential) are unpredictable, although a signature of the community's previous history is maintained. However, when the source communities are pre-conditioned to their new habitat, community development is more reproducible. This situation contrasts with some studies of communities of macro-organisms, where strong selection under novel environmental conditions leads to reproducible community structure, while communities under weaker selection show more variability. Our results suggest that the microbial rare biosphere may play an important role in the predictability of microbial community development, and that pre-conditioning may help to reduce unpredictability in the design of microbial communities for biotechnological applications.

Subject Category: Microbial population and community ecology

Keywords: Community similarity/ ecosystem development/ microcosms/ predictability/ rare biosphere

Introduction

Understanding the development of complex ecological communities is essential for effective biodiversity management and maintenance. It is particularly important to know to what extent community development can be predicted (and hence controlled) and, conversely, to what extent it is inherently unpredictable. This need is especially acute for microbial communities, which are key drivers of the Earth's biogeochemical cycles (Whitman *et al*., 1998), industrial processes including wastewater treatment, and human gut health (Palmer *et al*., 2007) and are the subject of ever-increasing datasets generated by modern DNA-based community analysis methods (Prosser *et al*., 2007; Prosser, 2012). Predictable factors such as environmental selection and interspecies interactions, as well as unpredictable factors such as random dispersal, stochastic population dynamics and priority effects (Chase, 2003; 2007), are all believed to affect microbial community structure and function (Langenheder and Székely, 2011; Prosser *et al*., 2007). However, the relative importance of these factors remains unclear.

Existing studies on microbial community assembly present conflicting views. Some studies suggest that, for a given set of environmental conditions, microbial community development is convergent: common environmental selection recruits the same or similar species from diverse starting species sets to produce similar final community structures (Figure 1; left panel). This view is supported by studies of sulphate-reducing bacteria in replicate sediment slurry microcosms (Kurtz *et al*., 1998), lab-scale activated sludge bioreactors inoculated with wastewater treatment plant communities (Ayarza and Erijman, 2011), soil community transplantation experiments (Lazzaro *et al*., 2011), microcosm colonization by rainwater bacteria

from different sites (Langenheder and Székely, 2011), and analysis of the predictability of seasonal changes in microbial community composition in the Pacific Ocean (Fuhrman *et al*., 2006). In contrast, other studies suggest that microbial community development is divergent: i.e. inoculation of identical replicate environments with the same initial microbial community results in different final community structures (Figure 1, right panel). This view is supported by studies of replicate aquatic microcosms inoculated from different environmental sources; after 3 weeks' development, the final communities were no more similar than the starting communities, and inter-replicate variation was high (Langenheder *et al*., 2006). Similar results have been reported for community development in simple hypolithic communities in desert environments (Caruso *et al*., 2011), wastewater treatment plants (Ofiteru *et al*., 2010), replicate laboratory phototrophic biofilms (Roeselers *et al*., 2006) and laboratory-scale wetlands (Baptista *et al*., 2008). It is important to note, however, that variable final community composition may be associated with stable broad-scale ecosystem function (Fernández *et al*., 1999; Langenheder *et al*., 2006).

For macro-organisms, there is some evidence that the relative importance of predictable and unpredictable factors in community development depends on the degree of environmental harshness (Chase, 2007) or disturbance (Violle *et al*., 2010). Pioneering work on the effect of drought on the assembly of macro-organisms in pond ecosystems (Chase, 2007) demonstrated that increasing the strength of environmental selection increases the reproducibility of the final community structure; this conclusion is supported by other studies of a variety of natural environments (Donohue *et al*., 2009; Helmus *et al*., 2010; Lepori and Malmqvist, 2009; Myers and Harms, 2011), as well as by microcosm studies of eukaryotic microbial (protist)

community assembly (Jiang and Patel, 2008; Weatherby *et al*., 1998). However, it is unclear whether these principles also apply to complex natural microbial communities. Microbial communities show much greater metabolic diversity than those of macro-organisms, and also typically possess a highly-diverse "rare biosphere" of species that are present at very low abundance (Pedrós-Alió, 2012; Sogin *et al*., 2006). Microorganisms also display shorter generation times, more rapid adaptive evolution and greater dispersal than macro-organisms. These factors may lead to a different disturbance-predictability relationship for microbial communities, compared to that of macroorganisms. Whether such factors can account for the apparently conflicting results on microbial community divergence or convergence is at present unclear. Interestingly, in simple microbial systems, evolutionary history has been shown to influence the diversification of individual species during community assembly (Knope *et al*., 2012).

In this study we consider whether the previous history of a complex microbial community affects the variability of its response to environmental selection. We address this issue using well-controlled laboratory experiments with replicated freshwater sediment-water microcosms. Microcosms provide a powerful tool for microbial ecology (Jessup *et al*., 2004), as they make it possible to perform replicate laboratory experiments under manipulable conditions. Many important advances have been made using "simple" microcosm communities with relatively few species (Hekstra and Leibler, 2012; Langenheder and Székely, 2011; Weatherby *et al*., 1998). In contrast, in this study we aim to preserve the key features of natural microbial ecosystems - high microbial diversity, nutrient cycling, community-environment feedbacks, strong inter-species interactions and spatial structure. Our system of

freshwater microcosms, based on the Winogradsky column (Madigan *et al*., 2011), retains these properties while allowing us to manipulate the extent of environmental selection or disturbance, to compare initial and final community composition and to measure community function in the form of a redox potential gradient.

In our experiments, groups of freshwater microorganisms colonize pristine (sterile) microcosm environments to form complex nutrient-cycling communities. By comparing final community composition and function among microcosms formed from the same and different source communities, we are able to test for convergence of initially different communities (Figure 1, left panel) or divergence of initially similar communities (Figure 1, right panel). Our results show that when colonization involves a drastic change in habitat (large environmental disturbance), the final community composition and function are unpredictable, with a high degree of variation between replicate experiments but retaining a signature of the source community. In contrast, when the community colonizes an environment to which it is already pre-conditioned, the final community composition can be more predictable.

Materials and methods

Sampling

Sediment and water samples were collected from 6 freshwater lochs and a freshwater pond in Scotland in September and October 2008 (Table S1). These sources cover a range of sizes (surface area 0.01 - 14 km^2), elevations (30 - 300 m), mean water pH values (6.16 - 8.70) and nutrient levels (Table S1). Sediment cores were taken from depths of 0.8 – 2.0 m using a hand-held corer, except at Loch Leven where a Jenkin's sediment sampler was used to sample from a depth of 3.8 m. Water and sediment sub-samples were then taken from the cores using sterile 50 ml Falcon tubes. Microbial diversity in these samples was characterised by Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting of the V3 regions of 16S rRNA genes from *Bacteria* and *Archaea* (Fernández *et al*., 1999).

Microcosms

Our pond sediment-water microcosms are based on the well-known Winogradsky column (Madigan *et al*., 2011). This system, originally designed to select for anaerobes, consists of a lower layer of pond sediment, mixed with nutrients (cellulose and sulphate) and an upper layer of water. Upon illumination for several weeks, the microcosm develops into a stratified, nutrient-cycling ecosystem. To create pristine microcosm environments, pond sediment and water from Blackford Pond in Edinburgh were sieved (1 mm pore size) to remove debris, and nutrients added: 0.25 g of $CaCO_3$ (buffer and CO_2 source), 2.5 g of cellulose (carbon source) and 5 g of $CaSO_4$ (sulphur source) per 100 g of sediment. The mixture was thoroughly homogenized and aliquoted into 15 ml Falcon tubes (7.5 g + 5 ml pond water per tube). The microcosms were then sterilized by autoclaving at 121 °C for 15 min and

stored at 4 °C. Triplicate sterilized microcosms were inoculated with 100 mg sediment and 100 μl water from the 7 environmental sites in a microbiological cabinet, before being thoroughly homogenized, then incubated under constant Northlight illumination in an incubator for 16 weeks at 25 °C, after which visible changes in the microcosms (e.g. colour changes) had largely ceased; additional tests demonstrate that redox potential and bacterial community structure also stabilise in this microcosm system by 16 weeks (Figure S1). Sterilised microcosms without inoculation did not develop redox potential gradients or colour changes when incubated under the same conditions for 19 weeks. For inoculation of "daughter" microcosms with pre-conditioned microcosm communities, two of eight mature (16 week) microcosms, originally set up using Blackford Pond sediment and water, were homogenized and 100 μl of the slurry were used to inoculate eight replicate sterilized microcosms, which were incubated as above.

Community DNA extraction and PCR amplification

Sediments from environmental samples and developed microcosms (sediment and water) were homogenized by vortexing. Community DNA was then extracted from 1 g sediment, 1 ml water or 1 ml homogenized microcosm using an UltraClean Soil DNA Isolation Kit (MoBio). Variable (V3) regions of bacterial and archaeal 16S rRNA genes were amplified for DGGE via a nested PCR approach using the primers and conditions listed in Table 1; *dsrB* gene fragments were amplified similarly but via a single round of PCR (Table 1). For 454 sequencing of bacterial 16S rRNA gene V3 regions from initial Loch Leven sediment, nested primers containing 454 adapter sequences (Table 1; Supplementary Methods) were used. All PCR reactions were set up in a PCR6 Vertical Laminar Airflow Cabinet with UV sterilisation (Labcaire

Systems Ltd.); both reaction tubes and PCR mixtures were treated for 15 minutes with 15 W UV light (wavelength = 254 nm) to destroy contaminating DNA, prior to addition of dNTPs, *Taq* polymerase and template DNA (Padua *et al*., 1999). Negative controls containing no added DNA template were routinely amplified alongside both rounds of functional PCR reactions.

Denaturing gradient gel electrophoresis (DGGE)

Nested PCR products were separated on DGGE gels (McCaig *et al*., 2001) containing a linear gradient of 30-70% denaturant [where 100% denaturant is defined as 7 M urea (42% w/v) and 40% (w/v) formamide]. For DGGE analysis of *dsrB* amplicons, a denaturant gradient of 40-80% was used. Electrophoresis was carried out on a DCode^{TM} Universal Mutation Detection System (Bio-Rad) in 7 L of 1 x TAE buffer at a constant temperature of 60 °C for 960 min at 75 V. Silver staining of gels was carried out as previously described (Nicol *et al*., 2005). All DGGE gels included 3 lanes with a standard set of 11 marker bands (see Supplementary Methods). DGGE fingerprints obtained from replicate PCRs of the same DNA extraction or replicate DNA extractions from the same microcosm were essentially identical (Figure S1).

Redox potentials

Redox potential gradients were measured using a heated platinum wire which was pierced into the side of the developed microcosms at 3 positions: in the upper part of the water layer, at the bottom of the sediment layer and 5 mm below the water-sediment interface. The potential of the wire was measured relative to a Ag/AgCl reference electrode (Radiometer Analytical), which was inserted into the top of the

microcosm water layer, using a WG020 voltmeter (Precision Gold) set at a sensitivity of 2 V.

DGGE fingerprint analysis

BioNumerics® Version 6.0 (Applied Maths) was used to produce normalised composite gels from the DGGE gels, using the marker lanes as a reference. Band-matching data with band intensities were imported into Primer 6 Version 6.1.12 (Primer-E Ltd.) and used to construct Bray-Curtis similarity matrices following square-root transformation to avoid domination by the most abundant species. The Bray-Curtis coefficient accurately captures diversity differences in various types of model datasets (Kuczynski *et al*., 2010). An index of 100 indicates identical fingerprints, while an index of 0 indicates no common bands (Clarke and Warwick, 2001). Nonparametric Multi-Dimensional Scaling (NMDS) plots generated from the Bray-Curtis similarity matrices in Primer 6 were used to represent the distance between each sample in two-dimensional space (Clarke and Warwick, 2001). NMDS was performed using 100 random starting configurations of sample points; the accuracy of the NMDS representation was determined by calculating the Kruskal stress (Kruskal, 1964). Primer 6 was also used for additional statistical analysis of the DGGE fingerprints and other parameters. ANOSIM and ANOVA (Clarke and Warwick, 2001; Sokal and Rohlf, 1969) were used to assess the significance of the source lochs in determining the observed similarity matrix and the mean microcosm redox potential respectively. PERMANOVA and PERMDISP analyses (Anderson *et al*., 2008) were carried out using the PERMANOVA+ add-on to Primer 6 to test for significant differences in the distribution and dispersion, respectively, of sets of communities based on Bray-Curtis similarities. For comparisons focusing only on

within-set variability, sets of replicate samples inoculated with different source communities were mapped onto a common centroid in multivariate space prior to carrying out PERMDISP analysis, in order to separate inter-replicate variation from variation between replicate sets (see Supplementary Methods). Similarity percentage (SIMPER) analysis (Clarke and Warwick, 2001) was used to investigate the contributions of individual bands (species) to the Bray-Curtis dissimilarity (100 - similarity) between the archaeal 16S rRNA gene fingerprints obtained from the microcosms.

Results and Discussion

Source communities

Source microbial communities comprised sediment and water samples from 6 freshwater lochs and a freshwater pond in Scotland (Table S1). The bacterial and archaeal sub-communities in these samples were characterised by DGGE fingerprinting of the V3 region of the respective 16S rRNA genes (Figure S2; see Materials and Methods). For the bacterial communities, the mean number of DGGE bands obtained from the water samples was 25.1 ± 1.7 (all error ranges quoted are ± s.e.m.) (Table S2; see also the Rr and $e^{H'}$ diversity measures in this table), while the sediment samples were so taxon rich that the resulting fingerprints could not be resolved. For the archaeal communities, the mean number of bands for the sediment samples was 26.7 ± 1.5 (Table S2); however archaeal V3 regions could not be amplified from the water samples, suggesting very low abundance of *Archaea* in the water. For the bacterial water communities and the archaeal sediment communities, the mean Bray-Curtis similarities were 41.6 ± 2.1 and 44.7 ± 1.8 respectively (Table S2). Therefore the source samples clearly differed in both bacterial and archaeal community composition. Similar biogeographical variation (Martiny *et al*., 2006) was seen for DGGE fingerprints of the bacterial dissimilatory sulphite reductase (*dsrB*) gene, specific to sulphate-reducing *Bacteria* (SRB) (Geets *et al*., 2006), in our samples (Table S2).

Colonization of a common, unfamiliar microcosm environment

Our 7 different source communities were allowed to colonize identical, pristine microcosm environments, under temperature and nutrient conditions very different from those in their source lochs, thus imposing strong environmental selection. Mixed

water and sediment from each source were used to inoculate identical triplicate microcosms. During incubation, a vertical redox potential gradient develops in the microcosms as a result of microbial activity, with less negative redox potentials in the upper layers of the microcosms (Table S3), indicating differences in the availability of electron acceptors at different levels of the stratified system. Both redox potential gradient and microbial community structure stabilise after 16 weeks' incubation (Figure S1). However, the final redox potential gradient varied considerably both among the microcosms (Figure 2), suggesting that different functional (redox) states can result from the community development process. There were significant differences in the vertically-averaged redox potential dependent on the source community [ANOVA, $F_{6,14} = 4.435$, $P = 0.01$]; however, as evident in Figure 2, there was also a large degree of functional unpredictability between replicate microcosms derived from the same inoculum. Consistent with this, the estimated contributions to the variability in redox potential due to source loch and inter-replicate variation were 43% and 57% respectively (Clarke and Warwick, 2001).

Unpredictable, but source-dependent, community composition following colonization of an unfamiliar environment

We next analysed the variability of the final community composition in our microcosms. As one might expect given the small size of the microcosms and the strong selection, community richness was substantially reduced compared to the inoculating sediments: mean band numbers were 22.8 ± 1.1 for bacterial 16S rRNA genes and 14.0 ± 0.7 for archaeal 16S rRNA genes (Figure S2), with similar decreases for Rr and $e^{H'}$ (Table S2). The lower $e^{H'}$ values also reflect a less even community composition in the microcosms compared to the inocula. SRB also showed reduced

diversity and evenness in the microcosm communities as measured by band number, Rr and $e^{H'}$ (Table S2).

DGGE analysis showed significant differences in composition between the microcosm communities after development in the common environment. Three aspects of the variation in community composition among our microcosms were notable. First, triplicate microcosms inoculated from the same environmental source showed low similarity (Figure 3): mean inter-replicate Bray-Curtis similarities were 57.3 ± 1.9 for *Bacteria* and 47.7 ± 4.1 for *Archaea* (Table S2), suggesting substantial unpredictability in community development. Second, despite this inter-replicate variability, the source community also influenced microcosm community structure: mean similarities between microcosms with *different* inocula were substantially lower (37.2 ± 0.5 for *Bacteria* and 27.4 ± 0.7 for *Archaea*; Table S2) than between those with the same inoculum. One-way ANOSIM tests (Clarke and Warwick, 2001) confirmed that the microcosm communities clustered according to source loch: $R = 0.914$, $p < 0.001$ for *Bacteria*; $R = 0.646$, $p < 0.001$ for *Archaea*; $R = 0.651$, $p < 0.001$ for SRB. PERMANOVA analysis also showed a significant effect of source loch on final microcosm community structure for all 3 genes (pseudo-F statistics between 2.98 and 4.47; $p = 0.001$; Table S2). Indeed, our PERMANOVA analysis showed that, for both *Bacteria* and *Archaea*, variability between replicate microcosm communities and effects of source loch made approximately equal contributions (in Bray-Curtis units) to the total variation among microcosm communities, while for SRB the variability between replicates was more important than the effect of source loch (Table S2).

A third interesting aspect of our results was that microcosm communities diverged in composition compared to the source communities. For *Archaea*, mean microcosm similarity was 30.3 ± 0.7 compared to 44.7 ± 1.8 for the initial sediment samples (Figure 3; Table S2), while for SRB the corresponding microcosm and sediment similarities were 18.9 ± 0.6 and 30.6 ± 1.9 respectively (Figure S3; Table S2). PERMDISP analysis (Clarke and Warwick, 2001) was used to test whether the dispersion between all microcosms was greater than that between inocula: this was true for both *Archaea* (F = 22.34; P = 0.002) and SRB (F = 12.91; P = 0.007; Table S2). Thus different source communities diverged, rather than converged, upon colonization of the common microcosm environment. For *Bacteria*, where only water samples, which do not contain most of the inoculum bacterial diversity, gave resolvable fingerprints, we could not perform this analysis.

Amplification of rare species upon community rearrangement in an unfamiliar environment

Differences in community composition between replicate microcosms could result from the random presence or absence of rare species in the inocula, if initially rare species are then amplified as the unfamiliar environment is colonized. To investigate this hypothesis, we compared the abundance of individual archaeal taxa in the source sediments and the developed microcosms. Indeed, many taxa that were undetectable in the source were abundant in the microcosms, and *vice versa* (Figure 4a) [defining "undetectable" as below the DGGE sensitivity threshold, which is estimated to be 1% of the total community (Muyzer *et al*., 1993)], suggesting that initially rare taxa are selected in our experiments. Even for those *Archaea* which were detectable in both the source and microcosm communities, we found no significant correlation between

initial and final abundance (Pearson r = -0.048; P = 0.104). For the bacterial community, where inoculating sediment fingerprints could not be resolved, we employed instead a high-throughput sequencing approach to study the initial sediment community. We obtained 10,277 pyrosequences of 16S rRNA gene V3 regions from the Loch Leven sediment sample (Supplementary Methods), and searched them for 28 sequences of cloned bacterial 16S rRNA gene V3 regions derived from the corresponding developed microcosms. Only 6 of these 28 sequences, which correspond to species which were abundant in the microcosms, were detectable (≥98% sequence identity) in the pyrosequencing dataset (Table S4). These results show that drastic community rearrangement occurred upon transfer to the unfamiliar microcosm environment, probably driven by strong selection for taxa different from those which were abundant in the source – many of which were rare in the source communities.

Interestingly, our analysis also showed that the selective effects on a given taxon during community development differed between replicate microcosms (Figure 4b) – individual archaeal taxa were amplified in some replicates but suppressed in others. For example, band no. 37 was amplified in two replicates of the Loch Tulla-derived microcosms compared to the inoculating sediment, but reduced in intensity in the third replicate (Figure 4b). It is also clear that many taxa were amplified in microcosms derived from some sources, but suppressed in microcosms from other sources. A similar effect was seen for individual SRB taxa (Figure S4). This suggests that there is no unifying environmental selection across the group of microcosms; rather, the complex dynamics caused by community-environment feedbacks and inter-species interactions, and/or stochastic effects, create different selective environments

in replicate microcosms. In our microcosms, metabolic feedbacks may lead to non-linear ecosystem dynamics, potentially producing chaotic or initial condition-dependent trajectories as observed in other studies (Becks *et al*., 2005; Benincà *et al*., 2008; Graham *et al*., 2007). *Indirect* interspecies interactions mediated by the physical environment are also likely to play a key role in our microcosms: for example, the generation of an anaerobic state as a result of cellulose degradation and sulphate reduction, which will strengthen selection for anaerobes in the developing community.

Does amplification of the rare biosphere cause unpredictability in community development?

To test for possible effects of rare species (undetectable by DGGE) on community divergence, we asked whether taxa which are rare in the source communities are also more variable in the final communities. Quantification of the contributions of individual archaeal taxa to the Bray-Curtis dissimilarity within triplicate groups of microcosms using SIMPER (see Materials and Methods) did not suggest increased contributions to microcosm dissimilarity for taxa which were undetectable in the source sediment (i.e. with zero DGGE intensity) compared to those which were detectable in the source (Figure S5). This does not, however, rule out the hypothesis that rare species are responsible for unpredictable community development. While the naïve view in which individual species make independent contributions to community variability clearly does not hold for our system, if interspecies interactions are strong then the random presence/absence of rare archaeal species in the inocula may lead to variability in the final abundance not only of those species but also of others, which were not rare in the inocula. The same analysis could not be performed for the

bacterial community due to the lack of resolvable 16S rRNA gene fingerprints from the inoculating sediments.

As another test for a causative role of the rare biosphere in unpredictable community development, we systematically decreased the size of the source community, by inoculating triplicate microcosms with serially-diluted Blackford Pond sediment and water samples (see Supplementary Methods). Microcosms seeded from smaller inocula should be more subject to the random presence or absence of rare species and therefore, according to our hypothesis, their final composition should be more variable (Langenheder *et al*., 2006; Wertz *et al*., 2007). Consistent with this hypothesis, the mean inter-replicate Bray-Curtis similarity index for *Archaea* in the developed microcosms decreased significantly from ~80 to ~40 over 4 orders of magnitude of dilution of the inoculum ($r = -0.55$; $P = 0.034$; Figure 5). For *Bacteria*, however, only a slight decrease in similarity, which was not statistically significant ($r = -0.43$; $P = 0.113$) was observed. Thus, while our data show that rare species are amplified and may play a role in causing unpredictability, the full story may be more complex. Ongoing high-throughput sequence analysis of the initial inocula and developed microcosm communities should assist in assessing this role.

Colonization of a familiar environment is more predictable

In our experiments so far, the source microbial communities experienced very different environmental conditions in the microcosms compared to those in their source lochs, leading to extensive community rearrangement and, probably, selection for initially rare species. We hypothesized that the results might be different if microcosms were colonized by communities which were already "conditioned" to the

microcosm environment of constant illumination, mesophilic temperature, high organic matter content and high sulphate – i.e. communities which do not undergo extensive rearrangement upon colonizing the microcosm. This can be achieved by using, as inocula, samples from homogenized mature microcosms.

To test this hypothesis, we set up eight replicate microcosms A-H (see Methods) derived from Blackford Pond sediment (we used eight replicates to provide better statistics). The developed microcosms showed significant variation in bacterial community composition (mean Bray-Curtis index = 34.2 ± 2.2; Table S2). Two of these pre-conditioned communities (A and H) were each then used to inoculate a further eight replicate microcosms, and the developed bacterial community compositions analyzed. As expected, community rearrangement was less extensive for the communities pre-conditioned to the microcosm environment than for those taken directly from Blackford Pond (Figure 6). For the pre-conditioned source communities, we observed significant correlation between the abundance of bacterial taxa in the source community and their abundance in the developed microcosm communities ($r = 0.452$, $P < 0.0001$ for source A; $r = 0.222$, $P = 0.002$ for source H; Figure 6b), in contrast to microcosms inoculated with environmental samples, where no correlation was observed (Figure 4).

To test whether community development was indeed more predictable when the microcosms were colonized by pre-conditioned communities, we calculated the average Bray-Curtis similarity between replicate microcosms seeded from the same pre-conditioned inoculum. This was 70.0 ± 1.2, substantially higher than in the original eight microcosms (34.2 ± 2.2; Table S2), a highly significant difference as

determined by PERMDISP (F = 89.03; P = 0.001)[a]. The NMDS plot (Figure 6a) also shows that groups of replicate microcosms seeded with the same pre-conditioned source community tend to cluster together with their source community, as demonstrated by a between-group mean Bray-Curtis similarity of 42.8 ± 0.8 (Table S2). This suggests an effect of community history stronger than that seen in the previous experiments. Thus, under conditions where the source communities are pre-conditioned to the microcosm environment, community development is more predictable.

An alternative explanation for our results could be that the variability of the final community correlates with the diversity of the starting inoculum, which is lower in our pre-conditioned communities. Random sampling of species from a more diverse (loch) source community might be expected to yield more variable inoculum communities than sampling from a less diverse (microcosm) community. However, although sediment archaeal communities are less diverse than those of *Bacteria*, we do not observe less variation of *Archaea* compared to *Bacteria* in our microcosms. Moreover, we do not observe a significant negative correlation between diversity of the source community and Bray-Curtis similarity of the resultant microcosm communities in our initial set of experiments (Figure S7a). To test the hypothesis further, we performed computer simulations of the sampling of inocula from species abundance distributions obtained from pyrosequencing datasets from Loch Leven

[a] We also compared the dispersion among replicate microcosms seeded from "pre-conditioned" Blackford Pond microcosm communities with that among replicate microcosms seeded with environmental samples from all the sources sampled. Here we found, in agreement with our hypothesis, that the mean Bray-Curtis similarity was greater for the former than the latter (57.3 ± 1.9 versus 70.0 ± 1.2; Table S2). However, the PERMDISP comparison showed only marginal significance (F = 3.56; P = 0.069).

sediment and from a Blackford Pond microcosm (Supplementary Methods; Figure S7b). For inoculum sizes corresponding to those in our experiments (greater than 10^5 individuals), there was little difference in the Bray-Curtis similarity between replicate simulated inocula from loch sediment and from the microcosm. Taken together, these analyses suggest that differential diversity of the source community is unlikely to be the sole explanation for differential microcosm variability in our experiments.

The picture presented in this work, in which colonization of an unfamiliar environment (and hence strong selection) leads to unpredictability, whereas colonization of a familiar environment is more predictable, allows us to rationalize the apparently conflicting results of previous studies on the predictability of microbial community assembly. When an immigrant community is subject to selection criteria very different from those it has previously experienced, as in the colonization of lab microcosms by aquatic microorganisms or colonization of desert habitats by globally-dispersed microbiota, we would expect unpredictable results, and indeed in these cases divergence of replicate communities is observed (Caruso *et al*., 2011; Langenheder *et al*., 2006). Conversely, if the colonizing community has experienced recent selection in a similar environment – as is likely to be the case for parallel bioreactors inoculated with wastewater treatment plant communities and soils transplanted between similar glacier foreland habitats - more predictable and convergent outcomes would be expected and are indeed observed (Ayarza and Erijman, 2011; Lazzaro *et al*., 2011).

Importantly, our results show variability between replicate communities at the functional level (assessed in this study by redox potential) as well as at the taxon

level. In our microcosms, as in environmental microbial communities (Burke *et al*., 2011; Trosvik *et al*., 2010), it is likely that the set of key microbial ecotypes that are present is reproducible across microcosms. However, our results suggest that variability at the taxon level can have a substantial effect on community function, even between replicate microcosms. The variation of broad-scale community function seen in our microcosms is greater than that observed in less spatially-heterogeneous and more short-term laboratory microcosm experiments (Langenheder *et al*., 2006). This may be because our system has strong, nonlinear feedback between community and environmental development: the final environment is largely created by the community itself. In this respect, our model system is similar to real-world microbial ecosystems, such as anaerobic bioreactors (Park *et al*., 2010) and the human gut (Palmer *et al*., 2007) in which the resident microorganisms are largely responsible for the prevailing environmental conditions.

While we have focused here on unpredictable amplification of rare species as a possible explanation for our observations of divergence between replicate communities colonizing unfamiliar environments, several other explanations are possible. The adaptive evolution of microbial species during our colonization experiments may also contribute to microcosm variability, with rare adaptive mutants playing a similar role to strongly selected taxa from the rare biosphere; evolutionary history can also influence the diversification of individual species in a simple community (Knope *et al*., 2012). The phenomenon of random, low-frequency exit from the non-growing state (Buerger *et al*., 2012) could also contribute to the amplification of different taxa in different replicate microcosms. Finally, the stochastic birth-death dynamics of low-frequency sub-populations (Black and

McKane 2012; Khatri *et al*., 2012), perhaps amplified by the effects of viral predation, may also influence the reproducibility of community development.

Conclusions

By allowing microbial communities sampled from different biogeographical sources to proliferate in replicate pristine microcosms, we have tested whether communities assemble in a predictable or unpredictable manner in a novel environment, and how this depends on the source community. When the community colonizes an unfamiliar habitat, replicate experiments produce very different outcomes, in terms of both community composition (for *Bacteria*, *Archaea* and SRB) and function (as measured by redox potential), and initially different inocula diverge rather than converge in composition. In contrast, when a pre-conditioned community colonizes a *familiar* habitat, community structure is more predictable. Our results show that on colonization of the unfamiliar microcosm environment, rare species, whose presence or absence is likely to vary randomly between replicate inocula, are strongly selected. When our communities instead colonize a familiar environment, the amplification of rare species is less prevalent, community reorganization is reduced and the final community composition is more predictable.

Our work sheds new light on the factors controlling the balance between predictability and unpredictability in microbial community development, and suggests new avenues for control of microbial community assembly. A host of biotechnological and biomedical applications, ranging from wastewater treatment to human gut health, depend crucially on our ability to predict and control the assembly of complex microbial communities from small inocula. Our results suggest that the design of engineered microbial communities is intrinsically limited by the effects of chance, but that pre-conditioning may prove a useful strategy for minimising the consequences of this unpredictability.

Supplementary information is available at ISME Journal's website.

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Titles and Legends to Figures

Figure 1 Alternative scenarios for microbial colonization of a novel, pristine environment. Left panel: replicate environments inoculated with different source communities converge towards similar final communities. Right panel: replicate environments inoculated with similar source communities diverge to give distinct final communities.

Figure 2 Community function within the developed microcosms differs between replicates. (**a**) Redox potential measurements taken in the developed (16 weeks) Loch Leven and Loch Lubnaig microcosms near the base of the sediment (2 cm), just below the sediment-water interface (6 cm) and near the top of the water layer (11 cm). The full set of data is presented in Table S3. (**b**) Microcosm redox potentials [means of the measurements at the 3 different heights in (**a**)] are shown for each of the 3 replicate microcosms from each source after 16 weeks' development.

Figure 3 Differences in community structure between the developed microcosms. NMDS plots of Bray-Curtis similarity between the DGGE fingerprints of bacterial (**a**) and archaeal (**b**) 16S rRNA genes from triplicate developed microcosms after 16 weeks' development show that triplicate microcosms differ in their final community compositions, while different source communities produce divergent microcosm communities. The corresponding fingerprints are shown in Figure S2b. For the archaeal communities the plot also shows the sediment inocula (circled); inoculum samples for bacterial *dsrB* genes are shown in Figure S3.

Figure 4 Archaeal taxon abundance in the source sediment does not correlate with that in the developed microcosms. Analysis of intensity of individual bands within the archaeal 16S rRNA gene DGGE fingerprints (**a**) shows no significant correlation between intensity in the sediment sample and intensity in each of the triplicate microcosms. (**b**) Selective effects on individual taxa differ between replicate microcosms. The difference in band intensity between the source sediment sample and each of the triplicate microcosms for each of the individual bands in (**a**) is shown. An example in which a single taxon is increased and decreased in abundance in different microcosm replicates (from the Loch Tulla source) is indicated. Colour codes are as in (**a**).

Figure 5 Effect of dilution of the inoculum on community similarity. A plot of Bray-Curtis similarity index (mean ± s.e.m.) of the bacterial and archaeal 16S rRNA gene fingerprints from the triplicate microcosms versus dilution factor of sediment + water inoculum. Dilution factor = 1 corresponds to the standard inoculum size used in other experiments. Best-fit trend lines and their slopes are indicated; the effect of inoculum dilution on similarity is significant for *Archaea*, but not for *Bacteria*.

Figure 6 Microcosm community composition is less variable for experiments with pre-conditioned communities. (**a**) NMDS plot of Bray-Curtis similarity between the DGGE fingerprints of bacterial 16S rRNA gene fragments from eight source microcosm communities (A-H) and the eight microcosms developed from sources A and H (labelled) shows that daughter microcosm communities are more similar to each other and to their source community (arrows) than to a different source or its daughter microcosms. The corresponding fingerprints are shown in Figure S6.

Analysis of the intensity of individual bands within the bacterial 16S rRNA gene DGGE fingerprints (**b**) shows a significant correlation between intensity in the source ("parent") microcosm and mean intensity in the replicate "daughter" microcosms from sources A and H (n = 8).